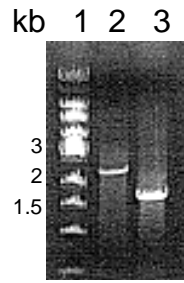


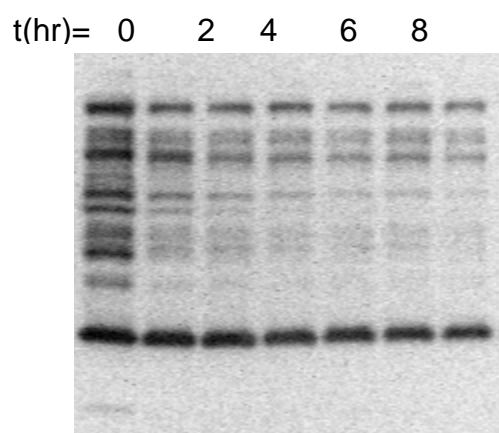
Supplemental figure 1. Tandem mass spectrometric data corresponding to the fragments of the ion observed in figure 3D.

Ion type	Obs ion	Obs Mass	Cal Mass	Error (Da)
B3	328.96	327.9527	328.14	-0.19
B4	476.25	475.2427	475.21	0.04
B5	575.15	574.1427	574.28	-0.13
B6	646.03	645.0227	645.31	-0.29
B7	717.4	716.3927	716.35	0.04
B8	864.39	863.3827	863.42	-0.04
B9	979.42	978.4127	978.44	-0.03
B10	1078.38	1077.373	1077.51	-0.14
B11	1193.38	1192.373	1192.54	-0.17
B12	1264.36	1263.353	1263.58	-0.22
B13	1635.48	1634.473	1634.76	-0.29
Y2	518.44	517.4327	517.28	0.16
Y3	589.44	588.4327	588.31	0.12
Y4	704.41	703.4027	703.34	0.06
Y5	803.38	802.3727	802.41	-0.04
Y6	918.40	917.3927	917.43	-0.04
Y7	1065.38	1064.373	1064.50	-0.13
Y8	1136.43	1135.423	1135.54	-0.12
Y9	1207.38	1206.373	1206.58	-0.20
Y10	1306.59	1305.583	1305.65	-0.06
Y11	1453.41	1452.403	1452.71	-0.31
Y12	1567.46	1566.453	1566.76	-0.30
Y6-G	861.21	860.2027	860.41	-0.21
Intact-GG	834.41	1666.80	1666.81	-0.01

Supplemental figure 2. PCR of WT and *prcBA* mutant using *pupfor* and *protmutr2* primers. Lane 1, DNA ladder; lane 2, WT; lane 3, *prcba* mutant.

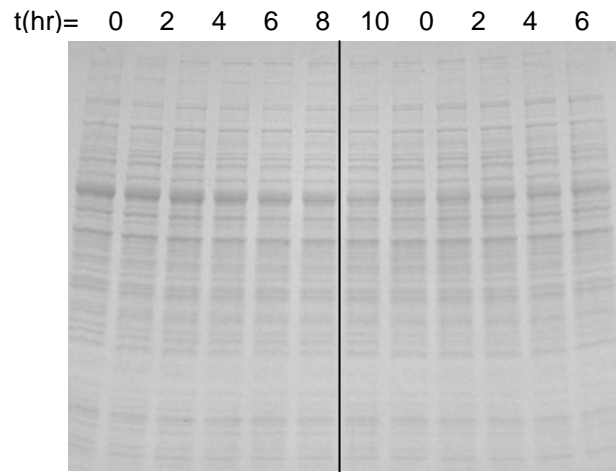


Supplemental figure 3. Decay of pupylated proteins in *prcba* mutant *M. smegmatis* complemented with a vector containing *prcba*, as observed by western blotting with penta-his-HRP conjugated antibody followed by chemiluminescence detection.



Supplemental figure 4. (A) SDS-PAGE analysis showing the decay of non-pupylated proteins in WT *M. smegmatis* extracts (left) and *prcbA* mutant extracts (right), as observed by Coomassie blue staining. (B) Quantification of (A) using ImageQuant (Amersham Biosciences). WT in triangles, *prcbA* mutant in squares.

A



B

